Genetic Analysis of Historical Human Remains –

What Ancient Bones can tell if questioned with optimized Methodology

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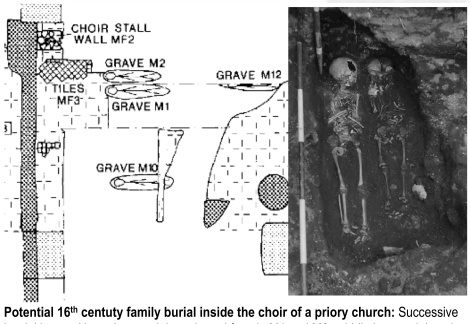
Ancient DNA analysis of historical human remains explores similar questions, utilizing similar and frequently the same methodology as applied in the forensic human identification context, while doing so under extreme conditions with regards to DNA content, degree of degradation and presence of inhibitors. Consequently, improvements of methodology and procedures in one of the areas will inform the other and vice versa. Our current Ancient DNA based research utilizes historical skeletal human remains from the collection of the Museum of Gloucester to investigate a variety of methodological questions in both the osteological as well as the DNA analysis context.



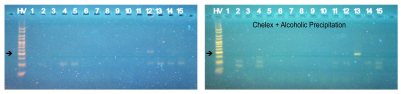
Potential 16th centuty family burial inside the choir of a priory church: Successive burrial layers. Upper layer: adult male and female M1 and M2, middle layer: adult male M3, bottom burials: 2 infants M4 and M5 (see picture, Ferris 2001).

As in the forensic context, sex determination and identification of individuals as well as kinship analysis involving group burials or across entire grave collectives are applications for DNA analysis-based methodology in the study of historical skeletal remains. The study presented here centers on a 16th century collective burial, excavated from the choir of a priory church in Gloucestershire England, which based on the relative location of the individual remains indicates a potential family burial: The group includes five individuals buried in overlaying consecutive horizons (Ferris 2001).

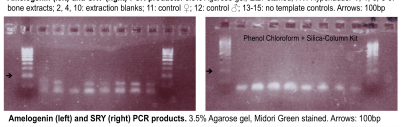
Analysis here utilized an STR multiplex of own design based on published medium amplicon primer sets (Kimpton et al. 1993), combined with molecular sex typing (Amelogenin: Sullivan et al. 1993, SRY: Santos et al. 1997). In a second phase this was followed up using single-plex amplification of autosomal STRs (VWA, TH01), Y-chromosomal STRs (DYS19, DYS393: Roewer & Ellplen 1992, Kayser et al. 1997) and the two sexing loci.

For DNA analysis, ca. 1g bone was sampled, surfaces were removed, the sample decontaminated and ground to a fine powder. Initially, DNA was extracted from 0.1g bone following a Chelex protocol with subsequent precipitation (Schmerer in prep.) after a 70h decalcification in 0.5M EDTA (pH 8.3). Subsequent amplifications showed the presence of remaining inhibitors in extracts from the historical remains (Deutschmann & Schmerer 2012, Cant 2014), which is a common problem in ancient DNA analysis (Höss & Pääbo 1993, Schmerer et al 1999).

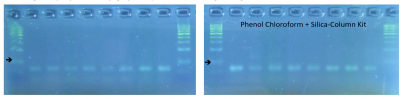
Initially, ribs were used whenever possible to minimize invasiveness of sampling. To improve outcomes, the second phase utilized 0.3g of samples with higher content of compact bone, replacement of EDTA solution after 24h and decalcification for 96h (Schmerer 2003), followed by phenol chloroform extraction (Schmerer et al 1999, Schmerer 2003) and purification of the aqueous phase by silica column-based extraction (Omega Biotek 2013).



Amelogenin (left) and SRY (right) PCR products. 3.5% Agarose gel, EtBr stained; HV: Hyperladder V; 1, 3, 5-9: bone extracts; 2, 4, 10: extraction blanks; 11: control ♀; 12: control ♂; 13-15: no template controls. Arrows: 100bp

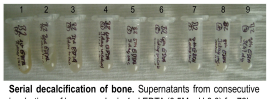


Amelogenin (left) and SRY (right) PCR products. 3.5% Agarose gel, Midori Green stained. Arrows: 100bp

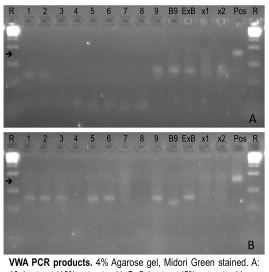


VWA PCR products. 3.5% Agarose gel, Midori Green stained. PCR: increased $MgCl_2$ concentration (2.5mM), 5µl extract (10% extract volume - left) and 3µl extract (6% extract volume - right). Arrows: 100bp

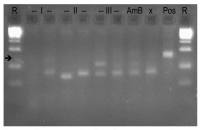
Previous attempts at genetic analysis indicated an extremely low degree of DNA preservation in the sample material, accompanied by a relatively high content of co-extracted inhibitors. Consequently, success in DNA amplification was rather limited, despite utilizing protocols optimized for the extraction of ancient DNA from historical human remains (Schmerer 2003), additional adaptation of protocols (Hunt et al 2016, Clemson 2016, Clemson et al 2017).and limited amplicon size of less than 200bp.



Serial decalcification of bone. Supernatants from consecutive incubations of bone powder in 1ml EDTA (0.5M, pH 8.0) for 72h



VWA PCR products. 4% Agarose gel, Midori Green stained. A: 10µl extract (10% extract vol.), B: 5µl extract (5% extract vol.).



VWA PCR products – purified and concentrated extract pool. 4% Agarose gel, Midori Green stained. I: 5µl, II: 2.5µl, III: 1.25µl pooled and Amicon-filtrated extract (10, 5, 2.5% extract pool vol.)

Extensive prior experience with DNA extraction from historical bone showed that repeated EDTA incubations of previously incubated bone can yield amplifiable DNA extracts. As long as residual bone powder is available, it will contain cellular material from which DNA can be extracted. Since DNA content of the material here proved to be extremely low, the latest phase of the study involves the development of an extraction protocol based on complete decalcification and dissolution of the bone powder, to utilize all genetic material embedded in the tissue. This was accomplished by repeated incubation of ca. 0.3g of bone powder in 1ml EDTA (0.5M, pH 8.0) for 72h at RT under constant agitation. The recovered supernatants showed continuous decrease in brownish colouring - indicative of the inhibitor humic acid - which is reflected in resulting inhibition patterns in amplifications (A).

Supernatants were subjected to DNA extraction using a silica-based kit (Omega Biotek 2017), following a modified version of the manufacturer protocol for body fluids. Aliquots of 10% (A) and 5% (B) of the resulting extracts were subjected to amplification at the STR locus VWA using a medium amplicon primer set (Kimpton et al. 1993). These showed that DNA content of the individual EDTA incubations of a sample is too low to yield sufficient product. Thus, 50% of the extract volume from each incubation of a sample were pooled and subjected to Amicom concentration (Millipore) and purification by washing in PCR grade water (UltraPure, Invitrogen). Aliquots of 10, 5 and 2,5% of the purified extract pool yielded detectable amplification product, but the drop-out patterns wthin the double-set indicate that DNA content is still at the trace level. Consequently, the next phase will utilize dentine samples, which due to the location of tooth root, enclosed by the bone of the jaw and its own density usually shows a higher preservation of the DNA contained and a lower content of inhibitory substances.

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